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The Development of Resistance to DDT in Musca Domestica L. Under the Effect of Sublethal Doses of Sodium Arsenate

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THE DEVELOPMENT OF RESISTANCE TO DDT IN *MUSCA DOMESTICA L.* UNDER THE EFFECT OF SUBLETHAL DOSES OF SODIUM ARSENATE

[Vozniknoveniye rezistentnosti k DDT u *Musca domestica L.* pod vliyaniye m subletal'nykh doz arsenata natriya]

V. P. Drobozina

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ABSTRACT. The possibility of development of resistance to organic contact insecticides belonging to the group of chlororganic (DDT) and phospho-organic (phenitrotion and chlorophos) compounds was studied when flies were given sodium arsenate, inorganic intestinal poison. Addition of sodium arsenate in sublethal doses to the food of imago of two susceptible strains of *M. domestica L.* in the course of 11 and 30 successive generations did not reduce the sensitivity of flies either to sodium arsenate or to phospho-organic insecticides. At the same time, in these experiments, a 200-fold increase of resistance to DDT was achieved with flies of both strains.

Development of resistance to DDT under the effect of sublethal doses of sodium arsenate occurred with variations in the level of resistance in first generations and permanent presence of a small portion of susceptible specimens even when a high level of resistance was achieved. This resistance to DDT is not associated with an increase of the intensity of dehydrochlorination of DDT and DDE.

Resistance to some insecticide in the absence of resistance to the insecticide used cannot be designated as "cross" or as "multiple." It is suggested that resistance of such type be called "side resistance."

THE DEVELOPMENT OF RESISTANCE TO DDT IN *MUSCA DOMESTICA L.* UNDER THE EFFECT OF SUBLETHAL DOSES OF SODIUM ARSENATE

V. P. Drobozina*

Data on the simultaneous resistance to a number of insecticides during the exposure of insects to one of these insecticides are noted in the literature (Lineva, 1963; Sukhova, et al., 1965; Yerofeyeva, 1962; El Bashier and Sawicki, 1963; Metcals and Georghiou, 1962). Many examples of such resistance are also cited in the survey of Frantsevich and Viktorov-Nabokov (1962), Hoskins (1967),** and Busvine (1967). Understanding the essence of this phenomenon, termed cross resistance, is very important for understanding the factors that cause the appearance of the latter. Here particular significance can attach to a study of the questions related to the development of simultaneous resistance to insecticides having different effects, as one of the manifestations of cross resistance that is hardest to explain.

In the study, we have attempted to clarify the development of cross resistance to contact insecticides — DDT and the phosphor-organic compounds (chlorophos and phenitrothion) — during the

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exposure of *Musca domestica L.* to sublethal doses of an intestinal poison from the group of inorganic compounds. We have used sodium arsenate for such a poison.

The possibility of insects developing resistance to the chlor-organic insecticides under the effect of an inorganic intestinal poison (sodium fluoracetate) was shown by the studies of Tahori (1963, 1965).

Material and Methods

We used flies of two sensitive strains for the experiments: normal and the standard SRS.* Over the course of a number of succeeding generations, sodium arsenate was added to the food of the imago (milk and glucose solution) of both strains. The same concentrations of poison were constantly used for the experiments — 0.005% for the SRS strain and 0.01% for the norm strain. The indicated concentrations, as our experiments showed, were the maximum sublethal concentrations for the imago of the parent generations. The level of sensitivity of flies to sodium arsenate was determined by the method of free feeding. Feeders containing a glucose solution that contained sodium arsenate in higher concentrations than those employed for daily feeding of flies, namely 0.01% to 1%, were placed for a day in the breeding cage holding 50 female and 50 male flies. Twenty-four hours later, the number of dead flies was counted and LC-50 was determined. Although, according to our observations, sodium arsenate is slightly repellant to flies, adding it to glucose and milk in the absence of other food baits induced the flies to drink it. This was shown not only by direct observations, but also by the fact that, with an increase in concentration of the insecticide, the mortality rate principally increases, but with a concentration of 0.5 — 0.75% sodium arsenate in the food, 90% or more of the flies die. This pertains equally to the first and subsequent generations of flies.

*All experiments were conducted with the SRS strain; the norm strain was used only to study the possibility of resistance to sodium arsenate and DDT.

Sensitivity of the experimental flies to the contact effect of DDT, chlorphos, and phenitrothion* was determined by the method of topically applying a methylethyletone solution of these insecticides by the aid of a syringe.

With the goal of clarifying the mechanism of resistance to DDT, the intensity of the dehydrochlorination process in males of several generations of the experimental strain SRS was determined. An index of the dehydrochlorination intensity was the proportion of DDT converted in the male organism to the nontoxic compound DDE over the course of a day following the application of 5 µg DDT along the mid back portion of the flies. A day after the application of DDT, 5 to 7 samplings of 3 to 5 specimens each were taken of the flies remaining alive. The amount of DDT and DDE were determined by the method of thin-section chromatography. The investigated substances were extracted from the flies using H-hexane; the extract was purified, and identified in a thin layer of aluminum oxide. Quantitative determination was carried out by measuring the areas of sample spots and standard solutions of the substances.

Sensitivity of the Experimental Strains to the Insecticides

We obtained 30 experimental generations of the SRS strain and 11 generations of the Norm strain. Long-term exposure to sublethal doses of sodium arsenate did not cause the appearance of resistance of the flies to this poison in any of the investigated strains of *M. domestica* (Figures 1 and 2, Table 1). The method employed by us for testing sensitivity (group feeding of flies using glucose containing sodium arsenate for a period of 24 hours) was such that we were unable to detect slight changes in sensitivity of the flies to the poison, but in any case, resistance to it did not develop.

*94.4% phenitrothion-(0,0-dimethyl-0-4-nitro-3-methylphenyl-thiophosphate) were employed.

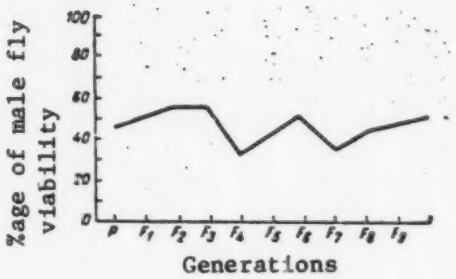

Figure 1. Viability of *M. domestica* of the Norm strain from exposure to a 0.075% concentration sodium arsenate over the course of the ten successive generations.

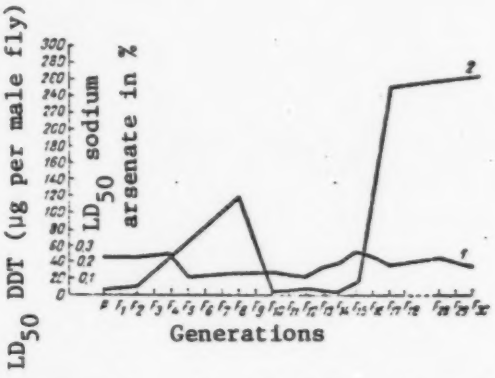

Figure 2. Sensitivity to sodium arsenate (LD_{50}) of *M. domestica* of the SRS strain over the course of 30 generations (1) and the development of resistance to DDT under the effect of low doses of sodium arsenate and *M. domestica* of the same strain (2).

The initial level of sensitivity of LD_{50} of the SRS strain to chlorophos and phenitrothion (0.8 and 0.1 µg for the males, respectively) practically remained unchanged throughout the course of the experiment (see Table 1). At the same time, the effect of sublethal doses of arsenic led to a significant increase in resistance to DDT in flies of both strains. The dose of DDT causing 50% lethality in the parent generations of the Norm strain was 0.9 µg for the male (45 µg/g), and for the SRS strain, 1.5 µg for the male (75 µg/g). LD_{50} for males of both strains, by the end of observations, have increased approximately 200 times, having reached 180 µg for the male (9000 µg/g) in the norm strain and more than 200 µg for the male (10,000 µg/g in the SRS strain (Figure 3).

The development of resistance to DDT under the effect of sublethal doses of sodium arsenate can be divided into three periods.

As can be seen from Figure 2, LD_{50} began to increase as early as the first generations of the SRS strain, and increased 80 times in F_8. Following this, however, a sharp drop in resistance occurred. In F_{10}, resistance of flies to DDT proved lower than the initial level by almost four times, LD_{50} during topical application

TABLE 1

SENSITIVITY OF *M. DOMESTICA* TO SODIUM ARSENATE, DDT, CHLOROPHOS AND PHENITROTHION OVER THE COURSE OF 30 GENERATIONS OF THE SRS STRAIN

Generation	Sodium Arsenate		DDT*		Chlorophos		Phenitrothion	
	Number of Experimental Flies	LC_{50} (in %)	Number of Experimental Flies	LD_{50} (in µg per male)	Number of Experimental Flies	LD_{50} (per µg per one male)	Number of Experimental Flies	LD_{50} (per µg per one male)
[illegible]	1200	0.23	800	1.5± 0.5	440	0.8	320	0.1
[illegible]	1200	0.23	800	2.0± 0.6	600	0.8	320	0.1
[illegible]	1200	0.23	800	10.0± 5.1	320	0.8	660	0.1
[illegible]	1200	0.25	800	47.0± 4.2	240	0.8	240	0.1
[illegible]	1200	0.16	[illegible]	[illegible]	240	0.8	240	0.1
[illegible]	1200	0.12	800	120± 20	280	0.8	240	0.1
[illegible]	1200	0.13	440	0.4± 0.1	280	0.8	280	0.1
[illegible]	1200	0.16	440	2.0± 0.3	240	0.8	320	0.1
[illegible]	1200	0.15	440	1.0± 0.4	240	0.8	280	0.1
[illegible]	1200	0.23	440	0.5± 0.1	240	0.4	280	0.06
[illegible]	1200	0.23	440	12.0± 2.7	200	0.5	280	0.06
[illegible]	1200	0.22	440	150± 20	200	0.4	280	0.06
F_{14}	600	0.17	400	>200	200	0.6	280	0.08
F_{15}	600	0.16	400	>200	200	0.6	280	0.07
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
F_{28}	600	0.23	400	>200	240	0.7	280	0.08
F_{29}	600	0.17	480	>200	240	0.7	280	0.08

*In the control, the level of resistance to DDT fluctuated from 1 to 2.4 µg/female.

comprised only 0.4 µg per male fly. The increased sensitivity to DDT was maintained over the course of five generations. But then, beginning with F_{15}, the level of resistance began rapidly to increase and in F_{17}, LD_{50} DDT reached 250 µg per male fly, and subsequently increased even more. The regression lines shown in Figure 4 for certain generations of the SRS strain show that, even with a high level of resistance (F_{17}, F_{28}), sensitive specimens are present in the strain: from 5 to 15% of the males died from doses of 0.05 to 0.5 µg per male specimen.

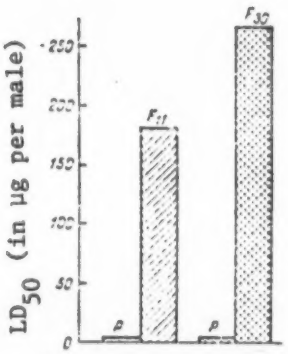

Figure 3. Level of resistance to DDT (LD_{50}) of the parent and subsequent experimental generations of two strains of *M. domestica* subjected to the effect of sodium arsenate.

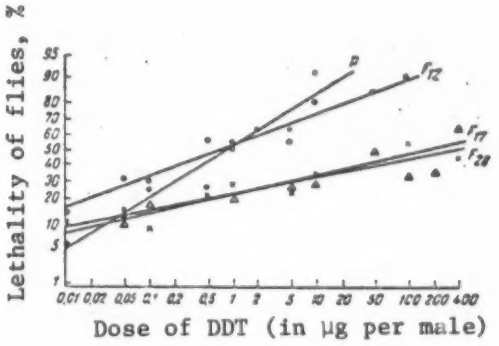

Figure 4. Change in the position of regression lines during the development of resistance to DDT under the effect of sodium arsenate in males of the SRS strain.

For studying the role of dehydrochlorination in the appearance of resistance to DDT under the effect of sublethal doses of sodium arsenate, we investigated the intensity of this process in male flies of the SRS strain in generations from F_{12} to F_{18}, i.e., in the period of transition of male flies of this strain from increased sensitivity to higher resistance to DDT. A certain amount of DDT converted in the flies' bodies over the course of a day into DDE showed that, with an increase in resistance to DDT occurring over the course of the indicated generations, the intensity of dehydrochlorination of DDT did not increase (Table 2). Moreover, flies of the same origin raised without poison and serving as the control were found to have approximately the same intensity of the DDT detoxification process. Hence, in the strain resistant to DDT that was obtained under the effect of sublethal doses of sodium arsenate, we did not succeed in detecting a parallel between the level of resistance to DDT and the activity of DDE formation. Apparently, resistance to DDT in this case was not due to dehydrochlorination, but to some other mechanism.

TABLE 2

INTENSITY OF THE PROCESS OF DDT DEHYDROCHLORINATION IN FLIES OF CERTAIN GENERATIONS OF THE SRS STRAIN

Generation of Flies	Amount of DDT Applied to the Experimental Flies (in µg)	Mean Amount of DDE Found (in µg)	Mean Percentage DDE Resulting from Applying the Amount of DDT	Level of Resistance to DDT (LD_{50}, in µg per fly)
F_{12}	15.0	5.9	39.3±2.9	2.0
F_{14}	15.0	4.2	28.2±2.3	0.5
F_{15}	25.0	9.4	36.1±1.8	12.0
F_{17}	25.0	9.6	38.4±3.4	>200.0
F_{19}	25.0	8.9	35.7±2.8	>200.0
Control	15.0	6.2		
	25.0	10.3	41.1±6.9	1.5

Discussion

The results of our investigation show that, during the exposure of flies to sublethal doses of inorganic intestinal insecticide, resistance to certain organic contact insecticides can appear, specifically, resistance to DDT. During this process, the development of resistance to DDT occurs unevenly, with an increase in sensitivity following an initial elevation of resistance, and also with constant maintenance of sensitive specimens, even upon reaching the maximum level of resistance in our experiments, i.e., similar to the way in which development of resistance to DDT resulting from chlorophos and diazine exposure occurs in *M. domestica* during the effect of sublethal doses of these insecticides (V. P. Derbeneva-Ukhova, 1960; V. P. Derbeneva-Ukhova, et al., 1966). This indicates a similar development of resistance under the effect of low doses of a poison, independent of the type of insecticide.

The basic mechanism of resistance to DDT in many species of insects, including *M. domestica*, is considered the enzymatic decomposition of this insecticide into a certain substance — DDE — with cleavage of HCl (Sternburg, et al., 1954; Abedi, et al., 1963; Kimura, et al., 1965, and others). Brown and Abedi (1960) found that this mechanism could also be responsible for the resistance to

DDT that developed under the effect of other insecticides. Thus, during breeding in the presence of malathion, resistance to DDT appeared in the house fly that was due to an increase in the activity of DDT-dehydrochlorinase. However, in the experiments of El Bashier and Lord (1965), the flies of a strain resistant to DDT produced very little DDE when subjected to the effect of diazinone.

The results of our investigation show that the process of DDT detoxification does not play a role in the given case with the development of resistance of DDT under the effect of sublethal doses of sodium arsenate.

There can be various reasons for the appearance of resistance to any insecticides under the effect of other poisons. It is suggested (Milani, 1963; Hoskins, 1967; Busvine, 1967; and others) that such resistance can either appear when the insect displays a single method of defense against two or more poisons, or with genetic factors which are adjacent in the chromosome that determine different mechanisms of resistance, such that breeding for one of them entails breeding for the others. In the former case, the term "cross" resistance is suggested, which was earlier used in all cases, and in the latter — "double, triple, or multiple" resistance.

Berim (1956) demonstrated a certain similarity in the action of DDT on insects with that of the arsenic compounds, and even a certain synergiotic effect. In our experiments, however, increased resistance to DDT in the experimental flies was observed without an increase in resistance to sodium arsenate.

Moreover, no correlation was observed between the level of resistance to DDT and the level of sensitivity to sodium arsenate (see Table 1), which refutes the existence of a single mechanism of resistance to DDT and sodium arsenate in the strains of flies investigated by us. For the same reason, one can also negate the second theoretically possible method of resistance to DDT — simultaneous selection of adjacent genes.

Hence, increasing resistance to DDT in *M. domestica* under the effect of sublethal doses of sodium arsenate cannot be explained by the reasons indicated above.

For the case of resistance described by us, the terms "cross" "multiple" resistance are not suitable. It seems to us that some other term must be used for designating this type of resistance, i.e., when resistance to the employed insecticide does not increase, but resistance to some other poisons does. It seems expedient to us to suggest the term "side" resistance. The case of "side" resistance discovered by us is not unique. As was already mentioned in the experiments of Tahori (1963, 1965), when breeding *M. domestica* in the presence of sodium fluoracetate, resistance to DDT and dieldrine appeared in the first generations, while only a weak tolerance to the employed preparation was observed. Resistance to a number of insecticides of the DDT group and dieldrine group — GKhTsG — frequently developed under the effect of the phosphor-organic compounds or carbamates, and is also frequently a "side effect" with respect to the employed preparations. For example, according to the observations of Lineva (1963) during the use of chlorophos against *M. domestica*, there was a significant increase in the level of resistance of the flies to DDT, while at the same time, sensitivity to chlorophos did not change.

"Side sensitivity," with respect to the employed insecticides, can simultaneously be "cross" or "multiple," if the employed poison causes resistance to certain insecticides. Thus, in the experiments of Meltzer (1958), breeding carried out in the presence of S-17 (N,N-dimethly-phenyl-carbaminate) led to the appearance of resistance to DDT, toxaphene, dieldrine, aldrine, chlordan, and lidan, as well as diazinone. At the same time, sensitivity to S-17 remained at approximately the same level (a resistance factor of 0.8 — 1.2). In this example, the resistance that appeared with respect to each of the preparations can be viewed as "side resistance" with respect to S-17 that was used. At the same time, simultaneous resistance to a number of related compounds can, in this case, be cross resistance, while with respect to the unrelated compounds, it can be multiple resistance.

As opposed to "cross resistance" and "multiple resistance," the reasons for the appearance of "side resistance" are unclear.

Conclusions

1. Constantly adding an intestinal poison — sodium arsenate — in sublethal concentrations to the food of *M. domestica imago* over the course of 30 successive generations did not cause the development of resistance to the employed poison, but did lead to an increase in resistance to DDT, by more than 200 times, in flies of two different laboratory strains.

2. The development of resistance to DDT under the effect of low doses of sodium arsenate occurred with fluctuations in the level of resistance in the first generation, with a constant maintenance of sensitive specimens, even upon reaching a high level of resistance.

3. The attained resistance to DDT is unrelated to an increase in the intensity of DDT dehydrochlorination.

4. Resistance to any insecticide in the absence of resistance to the employed insecticide cannot be termed either "cross resistance" or "multiple resistance." This type of resistance should be called "side resistance."

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